Novel Approach to the Synthesis of Isotopomeric Monoalkyl [^{16}O,^{17}O,^{18}O]Phosphates. The Stereospecific One-pot Conversion of (R_P)-Thymidine 3'-(4-Nitrophenyl)-phosphorothioate into (R_P)-Thymidine 3'-[^{16}O,^{17}O,^{18}O]Phosphate

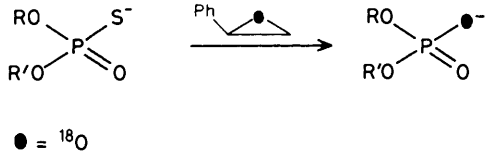
Andrzej Okruszek, Piotr Guga, and Wojciech J. Stec*

Polish Academy of Sciences, Centre of Molecular and Macromolecular Studies, Department of Bioorganic Chemistry, 90–362 Łódź, Boczna 5, Poland

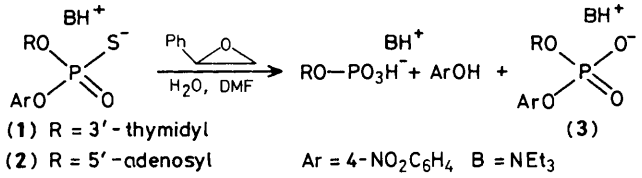
Reaction of the (R_P)-diastereoisomer of thymidine 3'-(4-nitrophenyl)phosphorothioate with styrene [^{18}O]oxide in a medium containing [^{17}O]water gives (R_P)-thymidine [^{16}O,^{17}O,^{18}O]phosphate in 70% yield with diastereoisotopomeric purity not lower than 92%.

It has been demonstrated in our previous report that oxiranes can be successfully used for a stereospecific conversion of *P*-chiral dialkyl phosphorothioates into the corresponding chiral [^{18}O]phosphates.[1] The reaction proceeds in moderate to high yields with retention of configuration at phosphorus (Scheme 1). Several *P*-chiral nucleoside [^{18}O]phosphates were synthesized in this way using styrene [^{18}O]oxide as the source of oxygen label.[2]

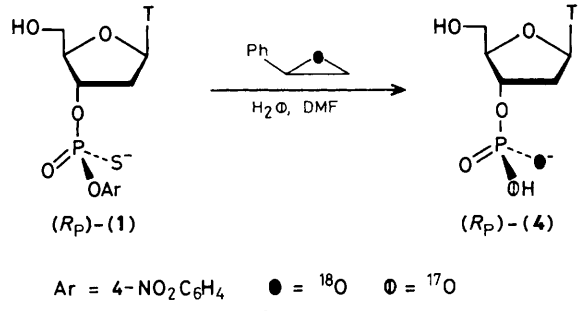
Continuing our studies on the interaction of phosphorothioates with epoxides we have found that if at least one aryloxy group is attached to phosphorus in the phosphorothioate molecule the reaction with oxiranes in aqueous medium proceeds in a different manner yielding the appropriate phenol and the corresponding phosphate as major products. When thymidine 3'-(4-nitrophenyl)phosphorothioate (**1**) and adenosine 5'-(4-nitrophenyl)phosphorothioate (**2**) were used as the substrates the corresponding nucleoside phosphates were obtained in 65—75% yield† (Scheme 2).



Scheme 1



Scheme 2



Scheme 3

† In addition to nucleoside phosphates and 4-nitrophenol *ca.* 15% of nucleoside (4-nitrophenyl)phosphate (**3**) is formed.

These observations prompted us to attempt the synthesis of *P*-chiral thymidine 3'-[^{16}O,^{17}O,^{18}O]phosphate (**4**) starting from diastereoisomerically pure (R_P)-(**1**),[3—5] using styrene [^{18}O]oxide[1,2] and [^{17}O]water as ^{18}O and ^{17}O donors, respectively (Scheme 3).

The reaction was performed on a 80 μmol scale with a 10-fold molar excess of styrene [^{18}O] oxide (91 atom% ^{18}O) in 450 μl of dimethylformamide (DMF) and 450 μl of [^{17}O]water (52.8 atom% ^{17}O, 41.8 atom% ^{18}O; Monsanto Research Corporation). The mixture was heated at 60 °C for 5 h and the product (**4**) was isolated in 70% yield by ion exchange chromatography (Sephadex A-25). Its identity and purity was confirmed by reverse-phase h.p.l.c.

The absolute configuration at phosphorus in (**4**) was determined according to procedure described by Lowe *et al.*[6] The chiral phosphate (**4**), after activation with diphenyl phosphorochloridate, was cyclised with Bu^{t}OK and then alkylated with MeI–Me_2SO–18-crown-6. The resulting mixture of axial and equatorial thymidine 3',5'-cyclic phosphate methyl esters (**5a,b**) was analysed by ^{31}P n.m.r. spectroscopy‡ at 121.5 MHz with a Bruker MSL-300 spectrometer (Figure 1). Comparison of spectroscopic data with calculated values (Table 1) clearly shows that 3'-[^{16}O,^{17}O,^{18}O]TMP was obtained with high diastereoisomeric purity (>92%)§ as the R_P-isomer.

Table 1. The relative peak intensities of the ^{31}P resonances in (**5a,b**).

	Equatorial triester (**5a**) Obs.	Calc.
MeO–P=O	0.33	0.16
Me●–P=O	1.00	1.00
MeO–P=●	0.60	0.50
Me●–P=●	0.44	0.39
	Axial triester (5b) Obs.	Calc.
MeO–P=O	0.30	0.16
Me●–P=O	0.56	0.50
MeO–P=●	1.00	1.00
Me●–P=●	0.39	0.39

‡ The equatorial assignment of the alkoxy substituent to a downfield ^{31}P n.m.r. signal in the family of 2-alkoxy-2-oxo-1,3,2-dioxaphosphorinane as well as in the nucleoside 3',5'-cyclic phosphate series is well documented: B. Maryanoff *et al.*, 'Topics in Stereochemistry,' Interscience Publishers, 1979, vol. 11, p. 187; J. Gerlt, 'Phosphorus-31 NMR,' Academic Press, London, 1984, p. 199; F. Eckstein *et al.*, *Biochemistry*, 1983, **22**, 1369.

§ This figure includes 6% loss of stereoselectivity during the cyclisation step.[6]

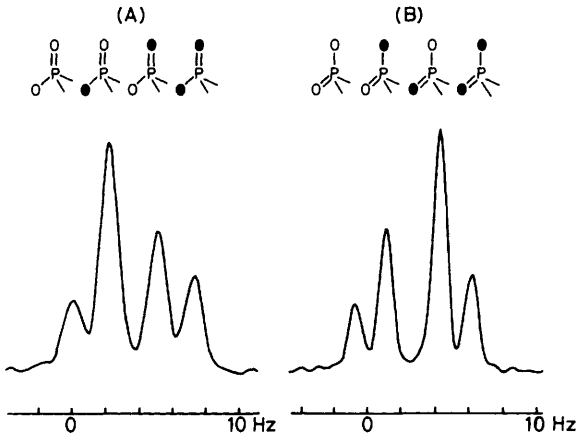
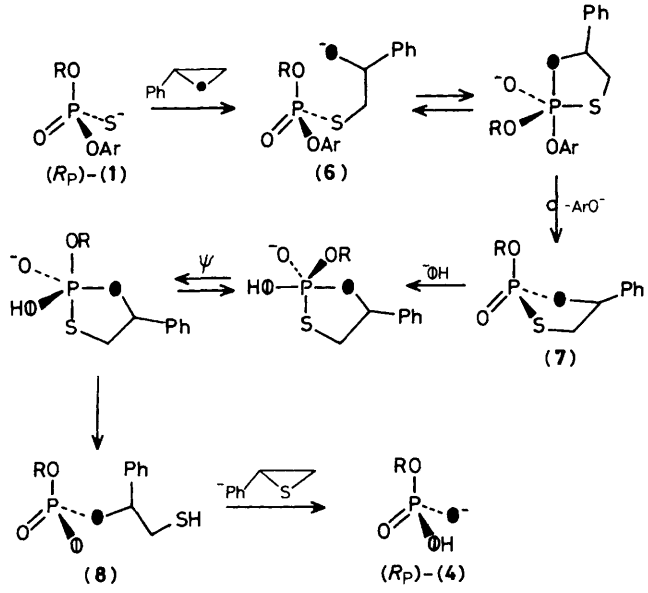


Figure 1. ^{31}P N.m.r. spectrum of the isotopomers (**5a**) and (**5b**). N.m.r. parameters: sweep width, 2000 Hz; pulse width, 3 μs; acquisition time, 2.2 s; Fourier transform in 32 K; line broadening 0.1. (A), equatorial ester (**5a**), δ (^{31}P) −4.93 p.p.m. (B), axial ester (**5b**), δ (^{31}P) −6.14 p.p.m.

This stereochemical result can be explained in terms of Hamer's classical mechanism[7,8] taking into account the good leaving group properties of the aryloxy substituent (Scheme 4). The intermediate (**6**) resulting from the initial addition of phosphorothioate anion to the oxirane molecule undergoes intramolecular rearrangement into the oxathiaphospholane (**7**) *via* nucleophilic attack of the vicinal hydroxy group at phosphorus with departure of the aryloxy group.¶ Compound (**7**) undergoes nucleophilic attack by water giving 2-mercaptoethyl phosphate (**8**) which spontaneously eliminates episulphide yielding the final phosphate product (**4**). It is assumed that substitution of the aryloxy group proceeds with inversion while the hydrolysis of the oxathiaphospholane, (**7**) → (**8**), occurs with retention of configuration at phosphorus. The latter process requires pseudorotation of a pentaco-ordinated intermediate. An alternative explanation of the stereochemical result, involving stereoretentive[1,2] transformation of (R_P)-(**1**) by means of styrene [^{18}O]oxide into (**3**; R = 3'-thymidyl) and subsequent stereoinvertive hydrolysis of (**3**) with [^{17}O]water, was ruled out since (**3**) was shown to be stable towards hydrolysis under conditions employed for the conversion (**1**) → (**4**).



Scheme 4

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